

## Amendments to the Specification

Please replace the first full paragraph on page 4 with the following amended paragraph:

The instant invention relates to a novel method, for use within serological or screening assays, wherein microbes are grown as colonies on filter membranes in multi-well plates, according to the following process. A sample containing a given microbe (bacteria, for instance) in a liquid (or other transferable) medium, is added to the wells of a multi-well filter plate, for example a Millipore™ Multiscreen™ MULTISCREEN 96 well filter plate (Millipore). Excess medium is then removed by a process of vacuum filtration, centrifugation or other suitable means and a nutrient source (in the form of a growth medium) is provided (e.g., THYE broth). Importantly, residual growth medium trapped in or under the filter membrane enables the growth of microbes in discrete colonies on the surface of the filter. Growth of the microbes in this manner allows for the colonies to be stained, imaged, and counted automatically using such automated systems as, *e.g.*, computer and video-based imaging systems. The assay can, thus, be exploited in evaluating the effectiveness of various antimicrobial agents on the growth and/or viability of various microorganisms in a timely and efficient manner.

Please replace the third full paragraph on page 6 with the following amended paragraph:

This was not appreciated prior to the instant invention. Filter membranes, to Applicants' knowledge, have been employed in the capture and isolation of microbes, and generally as a stage for various screening and serological assays, such as those carried out in multi-well plates. Upon completion of the reaction(s), however, the bacteria or tested microbe were transferred to a growth medium (*e.g.*, an agar petri plate), and manually counted in order to derive any experimental conclusions. Although devices exist for counting colonies on large (*e.g.*, 100 mm diameter) petri dishes, these devices are not generally amenable to rapid and automated counting of colonies grown in a multi-well (*e.g.*, 48 or 96 well) format. Efficient imaging and counting systems for assays run in multi-well plate format (*e.g.*, the C.T.L. imaging system for exemplified by the so-called ELISPOT assay such as that supplied by Autoimmun Diagnostika GmbH) have relatively recently become available. These imaging technologies, however, are not capable of enumerating microbial colonies grown on agar due to the

irregularities of the agar surface and the difficulties in uniformly plating multiple samples of microbes (e.g., bacteria) on a single plate.

Please replace the second full paragraph on page 7 with the following amended paragraph:

Filter plates of use in the instant invention are those suited for use in a multi-well format. The term “filter plates” employed throughout the instant application is to be interpreted as including both specifically crafted “filter plates” in multi-well format as well as simply 96 well plates comprising filters. Particularly preferred are Millipore™ 96 well plates, e.g., the 0.45µm (pore size) Durapore PVDF filters. Most preferred embodiments of the instant invention employ the Millipore™ MultiScreen™ 96 well plates such as the MULTISCREEN plates (Millipore). It is to be noted that the instant invention is not limited to wells contained within a 96 well format. Any multi-well format suited for ready analysis via automated means is definitely encompassed hereby. Such capabilities are enabled by Applicants’ finding that microbes can be manipulated to grow on filter membranes in multi-well format in distinct colonies to an extent comparable for vaccine evaluation purposes to microbes grown on agar.

Please replace the last full paragraph on page 8 with the following amended paragraph:

"As indicated above, following removal of medium from the wells, the microbe(s) are incubated for an amount of time sufficient to permit growth into discrete colonies for subsequent analyses; preferably, 14-18 hours, but generally dependent on the particular microorganism. Preferably, the plates comprising the microbes are kept hydrated. This can be accomplished through a number of means such as incubation in a humidified environment such as a water-jacketed humidified incubator or by covering the filter plate in, for instance, a Ziploc ZIPLOC bag (S.C. Johnson). Any suitable alternative serving to accomplish this same function is also encompassed hereby."

Please replace the second full paragraph on page 9 with the following amended paragraph:

After some time during which the microbe(s) are allowed to grow into discrete colonies on the filter plate, the colonies are fixed and stained; a preferred stain of which is Coomassie blue, but which can be any agent capable of providing a means for specifically highlighting an object for detection by the visualization, imaging and/or enumeration system employed. Staining in general terms enables ready visualization of the colonies by the system employed. The colonies are then analyzed. This is accomplished with any device suited for analysis of 96 well plates, or whichever multi-well format is utilized. Preferred for use in the instant invention is any computer-assisted video imaging and analysis system. In a particularly preferred embodiment, the computer-assisted imaging and analysis system is the IMMUNOSPOT ImmunoSpot™ Analyzer offered by (Cellular Technology Limited C.T.L. (Cleveland, OH)), or a similar imaging system offered by, for instance, Resolution Technology, Inc. (Columbus, OH).

Please replace the abstract with the following:

#### ABSTRACT OF THE DISCLOSURE

A method is provided, for use within serological or screening assays, wherein microbial colonies are grown on filter membranes in multi-well plates. This process enables the colonies to be stained, imaged, and counted automatically using such automated systems as, *e.g.*, computer and video-based imaging systems.